

EFFECT OF MANUFACTURING METHODS ON THE QUALITY OF YOGHURT

By
Ismael Mohammed Abdalla Adam
B.Sc. (Hon.) Animal Production
University of Gezira
(1999)

A thesis submitted in partial fulfillment for the requirements
of the degree of M.Sc. in Dairy Production and Technology

Supervisor
Dr. Mohamed Osman Mohamed Abdalla

Department of Dairy Production
Faculty of Animal Production
University of Khartoum

November 2008

DEDICATION

To my dear family,
Mother, brothers and sisters,
To my dear friends and colleagues

ACKNOWLEDGEMENT

All grateful to Allah for the assistance, health, patience and will to accomplish this work.

I would like to express my gratitude and thanks to my supervisor Dr. Mohamed Osman Mohamed Abdalla for his supervision, help and proper guidance during all the stages of this research.

I wish to thank my family for their valuable support and encouragement.

Also my thanks are extended to all members of Dairy Production Department, Faculty of Animal Production, University of Khartoum who helped me.

My thanks to everyone who helped me during the research.

LIST OF CONTENTS

Item	Page
DEDICATION	i
ACKNOWLEDGEMENT	ii
LIST OF CONTENT	iii
LIST OF TABLES	vi
ABSTRACT	vii
ARABIC ABSTRACT	viii
CHAPTER ONE: INTRODUCTION	1
CHAPTER TWO: LITERATURE REVIEW	3
2.1 Yoghurt	3
2.1.1 Definition of yoghurt	3
2.1.2 History of yoghurt making	3
2.1.3 Classification of yoghurt	4
2.2 Yoghurt manufacture	4
2.2.1 Pre-treatment of milk	5
2.2.2 Homogenization	5
2.2.3 Heat treatment (pasteurisation)	5
2.2.4 Cooling milk to inoculation temperature	6
2.2.5 Starter culture addition	6
2.2.5.1 Starter culture	6
2.2.6 Packaging of yoghurt	8
2.2.7 Incubation of yoghurt	8
2.2.8 Cooling of yoghurt	8
2.1.5 Chemical composition of plain yoghurt	9
2.1.6 Microbiology of plain yoghurt	9
2.1.7 Spoilage and pathogenic microorganisms in plain yoghurt	9
2.1.7.1 Yeasts	10
2.1.7.2 Moulds	11
2.1.7.3 Coliforms	11
2.1.8 The therapeutic value of yoghurt	12
CHAPTER THREE: MATERIAL AND METHODS	13
3.1 Collection and analysis of samples	13
3.1.1 Collection of samples	13
3.1.2 Analyses of Samples	13
3.1.2.1 Chemical analysis	13
3.1.2.1.1 Total solids content (TS%)	13
3.1.2.1.2 Fat content	14
3.1.2.1.3 Protein content	14

3.1.2.1.4 Solid not fat content (SNF%)	15
3.1.2.1.5 Titratable acidity	15
3.2 Microbiological examination	15
3.2.1 Preparation	15
3.2.1.1 Solid media	15
3.2.1.1.1 Violet red bile agar (VRBA)	15
3.2.1.1.2 Acidified potato dextrose agar (APDA)	16
3.2.1.1.3 Nutrient agar	16
3.2.1.2 Semi solid media	16
3.2.1.2.1 Hugh and Leifson medium (OF)	16
3.2.1.3 Liquid media	17
3.2.1.3.1 MRS broth	17
3.2.1.3.2 Brilliant green bile broth 2%	17
3.2.2 Sterilization	17
3.2.2.1 Sterilization of equipments	17
3.2.3 Culturing methods	17
3.2.4 Preparation of sample dilutions	17
3.2.5 Examination of culture	18
3.2.6 Examination of bacteria	18
3.2.6.1 Coliform count	18
3.2.6.2 <i>Lactobacillus bulgaricus</i> count	18
3.2.6.3 Yeasts and moulds count	18
3.2.7 Purification of organisms	18
3.2.8 Identification of organisms	19
3.2.8.1 Primary tests	19
3.2.8.1.1 Shape of the cell	19
3.2.8.1.2 Oxidase test	19
3.2.8.1.3 Catalase test	19
3.2.8.1.4 Oxidation fermentation test (OFT)	20
3.2.8.1.5 Fermentation test	20
3.3 Statistical analyses	20
CHAPTER FOUR: RESULTS	21
4.1 Chemical composition of plain yoghurt as affected by factory	21
4.2 Microbiological quality of plain yoghurt as affected by factory	21
4.3 Effect of storage period on the chemical composition of plain yoghurt	24
4.5 microbiological quality of plain yoghurt as effected by storage period (days)	27
CHAPTER FIVE: DISCUSSION	30

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS	33
6.1 Conclusion	33
6.2 Recommendations	33
REFERENCES	34

LIST OF TABLES

Item		Page
Table (2.1)	Proposed scheme for the classification of all yoghurt products	4
Table (2.2)	Chemical composition of plain yoghurt	9
Table (2.3)	Chemical properties of yoghurt	9
Table (4.1)	Chemical composition of plain yoghurt as affected by the factory	22
Table (4.2)	Microbiological quality of plain yoghurt as affected by the factory	23
Table (4.3)	Effect of storage period (days) on the chemical composition of plain yoghurt	25
Table (4.4)	The chemical composition of plain yoghurt within each factory as affected by storage period (days)	26
Table (4.5)	Microbiological quality of plain yoghurt as affected by storage period	28
Table (4.6)	The microbiological quality of plain yoghurt within each factory as affected by the storage period (days)	29

ABSTRACT

A study was carried to determine the chemical composition and bacteriological quality of plain yoghurt. Ninety samples of plain set yoghurt were collected from two factories in Khartoum State at day one of manufacture. Analysis of samples was carried out at 1, 5 and 10 day intervals.

The results showed that total solids, fat, solids-non-fat (SNF) contents and titratable acidity were significantly ($P=0.001$) different in the two factories. The highest total solids, fat and titratable acidity were found in factory 2, while the highest solids-non-fat content was found in factory 1. Protein content was non significantly affected by factory.

The storage period did not significantly affect total solids, fat, protein, solids-non-fat contents except for titratable acidity which was highly significantly ($P=0.001$) affected.

The *Lactobacillus bulgaricus*, coliform and yeasts and moulds counts were non significantly affected by factory, and their highest counts were in factory 1.

Storage period showed non significant effect on *Lactobacillus bulgaricus* and significant $P<0.05$ effect on coliform and highly significant ($P=0.001$) effect on yeasts and moulds counts of plain yoghurt.

المستخلص

أجريت دراسة لتحديد التركيب الكيميائي والمحتوى البكتيري للزبادي الذي يباع في ولاية الخرطوم. جمعت تسعين عينة من الزبادي البسيط المتماسك الخثرة من مصنعين في ولاية الخرطوم في اليوم الأول من تصنيع العينات. تم تحليل هذه العينات على فترات: في يوم التصنيع وبعد 5 أيام وبعد 10 أيام من تصنيع العينات.

أوضحت النتائج أن المواد الصلبة الكلية والدهن و المواد الصلبة اللادهنية والحموضة قد تأثرت معنوياً بالمصنع، وسجلت أعلى نسبة للمواد الصلبة الكلية و الدهن والحموضة في المصنع 2 بينما سجلت أعلى نسبة للمواد الصلبة اللادهنية في المصنع 1. أثبتت النتائج أن محتوى البروتين لم يتأثر معنوياً باختلاف المصنع.

أوضحت النتائج أنه لم يكن لفترة التخزين أي تأثير معنوي علي المحتوى من المواد الصلبة الكلية والدهن والبروتين والمواد الصلبة اللادهنية ما عدا الحموضة والتي تأثرت معنوياً بشكل ملحوظ.

كما دلت النتائج على أن أعداد البكتيريا العصوية اللبنية والبكتيريا القولونية والخمائر والفطريات لم تتأثر معنوياً بالمصنع وأعلى أعداد لها رصدت في المصنع 1. لوحظ ان تأثير فترة التخزين لم يكن معنوياً على البكتيريا العصوية اللبنية ولكنه كان معنوياً على البكتيريا القولونية والخمائر.

CHAPTER ONE

INTRODUCTION

Fermentation of milk was known around ten thousand years ago when man made the transition from food collection to food production and was a common method to extend the longevity of dairy products (Tamime and Robinson, 1991).

Fermented dairy products have been reported to be more nutritious than the milk from which they were made (Younus *et al.*, 2002).

Campbell-Platt (1987) defined fermented foods as those who have been subjected to the action of microorganisms or enzymes so that desirable biochemical changes cause significant modification to the food.

However, to the microbiologist, the term “fermentation” describes a form of energy-yielding microbial metabolism in which an organic substrate, usually a carbohydrate, is incompletely oxidised and an organic carbohydrate acts as the electron acceptor (Adams, 1990).

Milk can be fermented by bacteria, yeasts and filamentous fungi to produce a variety of products such as cheese, butter and yoghurt.

Yoghurt represents the most popular fermented milk product world-wide and originates from countries around the Balkan and the Eastern Mediterranean region (Staffe, 1998; Walstra *et al.*, 1999).

Yoghurt is derived from Turkish word “Jugurt” reserved for any fermented food with acidic taste. Yoghurt is prepared by fermentation of milk by usually two types of bacteria, namely *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Driessen, 1988; Marshall, 1993).

In set-style yoghurt, gels are formed (undisturbed) in the retail pot while stirred-type yoghurt is made by breaking the set gel before mixing with fruit and filling into retail containers (Tamime and Robinson, 1999).

Flavour, sour taste and aroma of yoghurt are due to the activities of most commonly mixed cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* which remain active in the product after its production (Bille and Keya, 2002; Douglas, 2005).

Some people suffer from lactose intolerance due to the fact that their digestive system lacks the enzymes which are needed to break down lactose to simpler sugars. In many cases cultured milk products where the lactose has been partially broken down can be acceptable to sufferers of lactose intolerance (Vesa *et al.*, 1996).

Yoghurt is a popular fermented milk product that has a special importance on the human health because of its perceived nutritional benefits. The probiotic elements in yoghurt are the lactic acid producing bacteria (LAB), which include *Lactobacillus* genera, *Bifidobacteria* and *Streptococcus thermophilus* have severally been demonstrated as the cause of their health benefits and the reasons for their description as functional foods (Adolfsson *et al.*, 2004).

Generally, yoghurt is manufactured from preheated milk. Fat and dry matter content vary with respect to region and legislation either in the plain form or with added materials.

Yoghurt production and consumption are rapidly increasing in Sudan, for this it is important that yoghurt quality, storage and transport instrument and temperature should comply with the Sudanese Standards for consumer protection.

The objectives of this study are:

- 1) Determination of the chemical and microbiological properties of plain set yoghurt from different factories.
- 2) Evaluation of the microbiological and chemical changes of plain yoghurt from different factories during the shelf life of 10 days.

CHAPTER TWO

LITERATURE REVIEW

2.1 Yoghurt:

2.1.1 Definition of yoghurt:

Yoghurt is a dairy food, produced by lactic acid bacterial fermentation of milk. The fermentation of milk sugar (lactose) into lactic acid gives yoghurt its characteristic gel-like texture (Binary Dictionary, 2005; Wikipedia, 2005; Elson and Hass, 2005).

Fermented milk drinks and yoghurts are dairy products produced from milk fermented with viable and retainable lactic acid bacteria (Adolfsson *et al.*, 2004).

Yoghurt is a semi-fluid milk product, prepared from fresh whole or skimmed milk, boiled and concentrated by evaporation, the fermentation is caused by the addition of culture bacteria and the thickness of the product is the result of the acidification by lactic acid bacteria (LAB) (Adams and Moss, 1995).

Yoghurt is a cultured dairy product produced by fermentation of milk with or without the addition of skim dry milk (NFDM). *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are the bacteria used as starter cultures (Lee *et al.*, 1990).

2.1.2 History of yoghurt making:

Fermentation is one of the oldest procedures for transferring raw materials of plant or animal origin into products, with that the fermentation of milk dates back approximately 10000 years (Stanley, 1998).

The use of yoghurt dates back many centuries, although there is no accurate record of the date when it was first made. According to legend, yoghurt was first made by the ancient Turkish people in Asia (Kurt, 1981).

2.1.3 Classification of yoghurt:

Tamime and Deeth (1980) have proposed a scheme of classification that separates all types of yoghurt into four categories based on the physical characteristic of the product. This approach is illustrated in the following table.

Table (2.1) Proposed scheme for the classification of yoghurt products.

Category	Physical state	Yoghurt product
I.	Liquid/ viscous	Yoghurt
II.	Semi-solid	Concentrated
III.	Solid	Frozen
IV.	Powder	Dried

Source: Robinson and Tamime (1990); Tamime and Marshall (1997).

According to Tamime and Robinson (1999) yoghurt is subdivided into different groupings based on the following aspects:

- 1) Legal standards (i.e. existing or proposed) to classify the product on the basis of chemical composition or fat content [Full fat, low fat and skimmed].
- 2) Physical nature of the product, i.e. set, stirred or fluid drinking. The latter is considered stirred yoghurt of low viscosity.
- 3) Post-fermentation processing (vitamin addition or heat treatment).
- 4) Flavours (plain/ natural, fruit or flavoured, the latter two types are normally sweetened).

2.2 Yoghurt manufacture:

Although there are no standardized procedures for making yoghurt product, most processors agree on a general process. This includes pre-treatment of milk, heat treatment, homogenization, cooling, starter culture addition, incubation and packaging (Tamime and Robinson, 1999).

2.2.1 Pre-treatment of milk:

The pre-treatment step involves adjusting the milk before processing. In set or stirred yoghurt production, this may include the addition of milk solids to achieve a desired viscosity. However, for a drinkable yoghurt milk without fortification is normally used (Tamime and Robinson, 1999).

Pre-treatment also includes the standardization of fat content in the milk. Therefore milk can be standardized in a variety of ways, this include part of the fat content may be removed from the milk, full cream milk may be mixed with skimmed milk, cream can be added to full fat or skimmed milk, or any combinatin of these using standardizing centrifuges (Tamime and Robinson, 1999).

2.2.2 Homogenization:

Is the next step in yoghurt manufacture also impacts the physical and chemical aspects of the milk. Homogenization breaks the large fat globules into smaller ones, thereby creating a stable emulsion of an oil-in-water mixture (Early, 1998; Spreer, 1998). This is typically accomplished by directing the stanardized milk through small valves at high pressure (Tamime and Robinson, 1999). The new fat globules are stabilized by binding to some of the casein that was broken during this processing step (Fox and McSweeney, 1998; Tamime and Robinson, 1999).

As a result, homogenization serves several purposes, including preventing the separation of the cream layer, improving stability, altering the physical attributes, the process ensures uniform mixing of any dry ingredients added to the milk (Tamime and Robinson, 1999).

2.2.3 Heat treatment (pasteurisation):

The primary reason for this heat treatment is the destruction of most pathogenic or potentially pathogenic microorganisms associated with milk (Early, 1998; Tamime and Robinson, 1999; Walstra *et al.*, 1999).

In commercial yoghurt production temperature-time profiles ranging from 80—85°C for 30 min or 90—95°C for 5 min are applied (Lucey and Singh, 1998).

Generally, heating conditions are much more intense than necessary for preservation purpose, causing a sufficient denaturation of whey proteins, which are then able to associate with casein micelles (Pearce, 1995; Law, 1996)

2.2.4 Cooling milk to inoculation temperature:

After pasteurisation the milk is cooled to the desired inoculation (starter culture addition) temperature typically 40 – 45°C (Tetra Pak Dairy Handbook, 1995).

2.2.5 Starter culture addition:

2.2.5.1 Starter culture:

The starter culture, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, are required for the fermentation in yoghurt production (Vedamuthu, 1991).

The classical yoghurt starter culture is a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, with a cocci-rods ratio of usually 1:1 (Hasan and Frank, 2001; Hutkins, 2001).

L. bulgaricus is a lactic acid bacterium that has been found to be relatively sensitive to low temperatures. According to Vedamuthu (1991) this microorganism is usually seen as rod shaped and although typically slender, may be curved or in pairs or chains.

As a homofermentative thermophile, *L. bulgaricus* is known to be relatively tolerant to heat, with an optimum growth temperature of approximately 45—50°C (Rasmussen, 1981; Vedamuthu, 1991; Rybka and Kailasapathy, 1995).

L. bulgaricus has strong tolerance for oxygen, therefore it is considered to be a relatively slow-growing organism, due to a lack of

oxygen. As a result of this, until the oxygen levels are reduced during fermentation, this microorganism will not grow rapidly (Marth and Steele, 1998).

The other starter microorganism involved in yoghurt production, *S. thermophilus* is a spherical-shaped, typically found in pairs or long chains and is noted for the ability to withstand higher temperatures, contributing to the classification of homofermentative thermophile (Vedamuthu, 1991). The thermal resistance is demonstrated in the evidence of *S. thermophilus* survival during heating at 60°C for 30 minutes and characterized by the use of these bacteria in various high temperature fermentations (Wilkins *et al.*, 1986^a). Despite being able to survive at higher temperatures, *S. thermophilus* fails to grow at 10°C, separating this microorganism from other streptococci that grow at lower temperatures (Marranzini, 1987). Other apparent attributes aside from optimum, minimum and maximum growth temperature, include the inability of *S. thermophilus* to grow in high salt concentrations and the inability to produce ammonia from arginine (Marth and Steele, 1998). These attributes are important for identification, since *S. thermophilus* does not possess a necessary antigen for serological identification and therefore, physiological techniques must be utilized (Marranzini *et al.*, 1989).

S. thermophilus and *L. bulgaricus* exist in a complex cooperative relationship in yoghurt in which one bacterium produces stimulatory agents for the other (Tamime and Deeth, 1980; Wilkins *et al.*, 1986^b; Schmidt *et al.*, 1989; Vedamuthu, 1991).

L. bulgaricus has been shown to produce certain amino acids such as valine, leucine and histidine, which are essential for *S. thermophilus* to grow. These amino acids are the result of proteolysis of casein by *L. bulgaricus* (Marranzini *et al.*, 1989; Abu-tarboush, 1996).

S. thermophilus in turn encourages the growth of *L. bulgaricus* by producing formic acid and carbon dioxide (Matalon and Sandine, 1986; Rajagopal and sandine, 1990).

2.2.6 Packaging of yoghurt:

Packaging of yoghurt is an important step during production and the purpose of packaging can be summarized as follows:

- 1) Protection of product from dirt, microorganisms and enviroment, e.g. gases (oxygen) and light.
- 2) Provide relevant information to the consumer, e.g. the food labeling guidelines: name and origin of the food, ingredients, instruction for use and expiry date.
- 3) The packaging material must be non-toxic and no chemical reaction should take place between the material and the product (Tamime and Robinson, 1985).

2.2.7 Incubation of yoghurt:

The conditions of incubation may additionally influence the properties of the final product. Generally, thermophilic lactic acid bacteria show an optimum temperature ranging from 40 to 43°C. It was generally accepted that the lower the fermentation temperature, the longer it takes to reach a certain pH and therefore firmness, but the final product is much firmer (Walstra *et al.*, 1999). However, Lankes *et al.* (1998) compared yoghurt manufactured at either 30°C or 42°C and found higher gel firmness and higher viscosity for products fermented at 42°C.

2.2.8 Cooling of yoghurt:

Once yoghurt has reached the desired a pH of 3.9 (Marshall, 1993), it is refrigerated to induce the bacteria into a dormant state to halt acid production.

At the required acidity (around 0.8—1.0%) lactic acid cooling of the coagulum commences and the intention is to reduce the temperature of the

coagulum to below 20°C with an acceptable time span (Robinson, 1981). Thus below 20°C the metabolic activity of the starter organisms is sufficiently reduced to prevent yoghurt from becoming unpalatable through excess acidity and hence initiation of cooling depends on the level of lactic acid required in the end product (usually between 1.2—1.4% lactic acid). The rate of cooling that can be achieved with the available equipment, and in a manner that does not damage the texture of yoghurt (Robinson, 1981).

2.1.5 Chemical composition of plain yoghurt:

The major components of yoghurt are in the following table:

Table (2.2) chemical composition of plain yoghurt.

Components%	Traditional yoghurt
Fat	5.54
Protein	4.64
Total solids	15.82
Lactose	4.1

Source: Ibrahim *et al.* (1992)

According to the Sudanese Standards (2007) the chemical properties of yoghurt were illustrated in the following table.

Table (2.3) chemical properties of yoghurt.

Yoghurt types	Minimum fat content%	Maximum fat content%	Minimum SNF content%
Full fat yoghurt	3	—	8.2
Partially skimmed yoghurt	0.5	>3	8.2
Skimmed yoghurt	—	0.5	8.2

2.1.6 Microbiology of plain yoghurt:

Yoghurt is prepared by fermentation of milk by usually two types of bacteria, namely *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Driessen, 1988; Marshall, 1993) and all other microorganisms present should be considered contaminants (Moreira *et al.*, 2001).

2.1.7 Spoilage and pathogenic microorganisms in plain yoghurt:

Yeasts and moulds are the main spoilage organisms found in cultured milk (yoghurt, sour cream and butter milk) because high acidity in these products inhibits many bacteria (Viljoen *et al.*, 2003; Mayoral *et al.*, 2005).

Numerous studies (Roostita and Fleet 1996; Gadaga *et al.*, 2000; Carbo *et al.*, 2001; Gadaga *et al.*, 2001) linked the increasing presence of yeasts and moulds in fermented dairy products to insufficient hygiene during the production and sanitation of the equipments, air-contamination, insufficient heat treatment, or inadequate microbiological quality of the supplements used.

Abdalla and El Zubeir (2006) found high counts of *E. coli*, *Staphylococcus aureus*, and *Salmonella* spp. in whole milk, skim milk, yoghurt and their mixtuer and reported that these organisms are a potential food spoilage. *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Yersinia enterocolitia* are three of the most important foodborne bacterial pathogens and can lead to foodborne diseases through consumption of contaminated milk and fermented milk products including yoghurt (Morgan *et al.*, 1993; Mead *et al.*, 1999).

According to the Sudanese Standards (2007) yoghurt should be free of pathogenic bacteria, bacteria toxins and the spores bacteria should be less than 10 cfu/gm.

2.1.7.1 Yeasts:

Yeasts are a subset of a large group of organisms called fungi that also include moulds and mushrooms. One of the most common yeasts in fermentative food spoilage is *Zygosaccharomyces rouxii*, which is able to grow at as low as 0.62% lactic acid and at pH values between 1.5 and 10.5 (Restaino *et al.*, 1983; James and Stratford, 2003).

The spoilage of yoghurt by yeasts results in defects manifested by changes in the yoghurt texture, yeasty, fruity or bitter flavour, and by unpleasant off-flavour (Robinson and Tamime, 1990; Viljoen and Greyling, 1995).

Some dairy products like cream, butter, cheese and yoghurt are likely to be spoiled by yeasts (Fleet, 1990; Addis *et al.*, 2001; Viljoen, 2001; Cocolin *et al.*, 2002). The presence and predominance of certain yeast species in these products are related to their physico-chemical properties. *Candida spp.* and *Kluyveromyces spp.* are the main yeasts isolated from plain yoghurt (Fleet, 1992) whereas fruit yoghurt, due to the addition of sugar or fruit is also prone to spoilage caused by *Saccharomyces cerevisiae* (Viljoen, 2001).

Contamination by yeasts is one of the main limiting factors for the stability and the commercial value of yoghurts (Canganella *et al.*, 1998; Viljoen *et al.*, 2003).

Spoilage becomes evident when yeast population reaches 10^5 to 10^6 cells g^{-1} (Fleet, 1992; Loureiro and Querol, 1999).

2.1.7.2 Moulds :

Moulds are filamentous fungi that do not produce large fruiting bodies like mushrooms. Moulds are very important for recycling dead plant and animal remains in nature but also attack a wide variety of foods and other materials useful to humans. Most moulds grow at a pH range of 3—8 and some can grow at a very low water activity levels (0.7—0.8) on

dried foods (Pitt and Hocking, 1997). Some spoilage moulds are toxigenic and capable of producing toxic metabolites known as mycotoxins causing serious public health concern, while others are not (Pitt and Hocking, 1997).

Aflatoxins have been demonstrated as potent human carcinogenic, mutagenic and tetragenetic. They are highly stable during processing and storage of yoghurt (Kivanc, 1992; Egmond, 1994; Roy *et al.*, 1996; Shibario *et al.*, 1998; Hassanin, 1999; Galvano *et al.*, 2000; Mishra and Das, 2003 ; Elena *et al.*, 2004).

2.1.7.3 Coliforms:

Coliforms may be defined as gram-negative, oxidase-negative, nonsporing rods which can grow aerobically or facultative anaerobically in the presence of bile salts or other surface-active agents with similar growth inhibiting properties and which are able to ferment lactose with production of gas within 48 hr at 37°C (Lovell, 1990).

According to the Turkish Standards Institute (TS1330) a maximum count of 10 cfu/gm of coliform is allowed in yoghurt (Anonymous, 1989). In yoghurt coliform bacteria are present as contaminants and may be used as indicators of sanitary conditions.

2.1.8 The therapeutic value of yoghurt:

Human consumption of yoghurt has been associated with tremendous health benefits due to improvement of gastrointestinal functions and disease risk reduction (Heyman, 2000). These health benefits include improved lactose digestion and elimination of lactose intolerance symptoms among maldigesters (Vesa *et al.*, 1996), lowered cholesterol level and reduction in the risk of hypertension (Takano, 1998; Taylor and Williams, 1998), lowered cancer tendency (van't Veer *et al.*, 1989), diarrhea prevention and control as well as maintenance of gastrointestinal microflora (Boudraa *et al.*, 1990).

CHAPTRE THREE

MATERIALS AND METHODS

3.1 Collection and analysis of samples:

3.1.1 Collection of samples:

Ninety samples of set yoghurt (45 samples from Zadi factory and 45 samples from Algota factory) were collected during the period from June to December 2007. The samples were collected at day 1 of manufacture and were kept at refrigeration temperature (7°C) and analyses were carried out at 1, 5 and 10 days intervals.

3.1.2 Analyses of samples:

3.1.2.1 Chemical analysis:

3.1.2.1.1 Total solids content (TS%):

Total solids content was determined according to the modified method of AOAC (2003) as follows:

Three grams were weighed in dry clean flat-bottomed aluminum dish and heated on a steam bath for 10 minutes.

The dishes were then placed in an oven at 100°C for three hours, then cooled in a desiccator and weighed quickly. Heating, cooling and weighing were repeated until the difference between the two readings was less than 0.1 mg. The total solids (TS) was calculated using the following equation:

$$TS\% = W_2 / W_1 \times 100$$

Where:

W_1 : weight of sample before drying.

W_2 : weight of sample after drying.

3.1.2.1.2 Fat content:

Fat content was determined by Gerber method according to AOAC (2003) as follows:

In a clean dry Gerber tube, 10 ml of sulphuric acid (density 1.815 gm/ ml at 20°C) were poured and then 11gm of a well mixed yoghurt sample was gently added. One ml of amyl alcohol (density 0.814—0.816 gm/ml at 20°C) was added to the mixture, the contents were then thoroughly mixed till no white particles could be seen. Gerber tubes were centrifuged at 1100 revolutions per minute (rpm) for 4 minutes and the tubes were then transferred to a water bath at 65°C for 4 minutes. The fat percent was then read out directly from the fat column.

3.1.2.1.3 Protein content:

Protein content was determined according to Kjeldahal method (AOAC, 2003).

In a dry clean Kjeldahal flask, 11 gms of yoghurt were added, then 25 ml of concentrated H_2SO_4 were added followed by addition of two Kjeldahal tablets (CuSO_4). The mixture was then digested on a heater until a clean solution was obtained after 3 hours. The flasks were removed and left to cool. The digested sample was poured into a volumetric flask (100 ml) and diluted to 100 ml with distilled water. Then 5 ml were taken, neutralized using 10 ml of 40% sodium hydroxide (NaOH) and the neutralized solution was then distilled. The distillate was received in a conical flask containing 25 ml of 4% boric acid plus three drops of indicator (bromocresol green plus methyl red). The distillation was continued until the volume in the flask was 75 ml. The flask was then removed from the distillator, and the distillate was then titrated against 0.1N HCl until the end point was obtained (red color).

The protein content was calculated as follows:

$$\text{Nitrogen (\%)} = \frac{T \times 0.1 \times 0.014 \times 20 \times 100}{\text{Weight of sample}}$$

$$\text{Protein (\%)} = \text{Nitrogen} \times 6.38$$

Where:

T: Titration figure (ml).

0.1: Normality of HCl.

0.014: Atomic weight of nitrogen/ 1000.

20: Dilution factor.

3.1.2.1.4 Solids not fat content (SNF):

The solids not fat was obtained by subtracting fat from total solids as follow:

$$\text{S.N.F\%} = \text{T.S\%} - \text{Fat\%}.$$

3.1.2.1.5 Titratable acidity:

The titratable acidity was determined according to Bradley *et al.* (1992).

Nine grams of mixed yoghurt sample were weighed into a clean beaker (100 ml) by using a clean pipette. Five drops of phenolphthalein indicator (1%) were added and titrated with 0.1N Sodium hydroxide (NaOH) to the first permanent color change to pink, then the percentage of lactic acid was calculated using the following equation:

$$\text{Acidity (\%)} = \frac{(\text{ml NaOH}) \times (\text{N NaOH}) \times 9}{\text{Weight of sample}}$$

Where:

NaOH: Sodium hydroxide.

9: Conversion factor for lactic acid.

3.2 Microbiological examination

3.2.1 Preparation of media

3.2.1.1 Solid media

3.2.1.1.1 Violet red bile agar (VRBA):

The medium consisted of 7 gm peptic digest of animal tissue, 3 gm yeast extract, 10 gm lactose, 1.5 gm bile salts mixture, 5 gm sodium chloride, 0.03 gm neutral red, 0.002 gm crystal violet and 15 gm agar.

The medium was prepared by dissolving 41.53 gm of powder in one liter of distilled water, followed by boiling to dissolve the medium completely. This medium is heat sensitive, therefore the excessive or prolonged heating was avoided during dissolving and was not autoclaved because autoclaving reduces the medium productivity (Jensen and Hausler, 1975; Hartman and Hartman, 1976).

3.2.1.1.2 Acidified potato dextrose agar (APDA):

The medium consisted of 200 gm potatoes infusion form, 20 gm dextrose and 15 gm agar.

The medium was prepared by dissolving 39 gm of powder in one litre of distilled water, followed by boiling to dissolve the medium completely and it was sterilized by autoclaving at 121°C for 15 minutes and cooled to 46°C, then enough sterile 10% tartaric acid was added.

3.2.1.1.3 Nutrient agar:

This medium consisted of 5 gm peptic digest of animal tissue, 1.5 gm beef extract, 1.5 gm yeast extract, 5 gm sodium chloride and 15 gm agar.

The medium was prepared by suspending 28 gm of powder in one liter of distilled water, then boiled until dissolved completely and sterilized by autoclaving at 121°C for 15 minutes.

3.2.1.2 Semi solid media:

3.2.1.2.1 Hugh and Leifson medium (OF):

This medium consisted of 2 gm peptone, 5 gm sodium chloride, 0.3 gm dipotassium hydrogen phosphate, 3 gm agar and 0.2% bromothymol blue. It was prepared according to Barrow and Feltham (1993) by dissolving the solid components in one liter of distilled water, then 15 ml of bromothymol blue (0.2% aqueous solution) indicator were added. The medium was sterilized by autoclaving at 121°C for 15 minutes, then sterile solution of glucose was added till it has a final concentration of 1%. The medium was then mixed well and distributed aseptically into sterile tubes.

3.2.1.3 Liquid media:

3.2.1.3.1 MRS broth:

This medium consisted of 10 gm proteose peptone, 10 gm beef extract, 5 gm yeast extract, 20 gm dextrose, 1 gm polysorbate (80), 5 gm sodium acetate, 0.10 gm magnesium sulphate, 0.05 gm manganese sulphate and 2 gm dipotassium phosphate. Fifty five grams of media were dissolved in one liter of distilled water, it was mixed well and then autoclaved at 121°C for 15 minutes.

3.2.1.3.2 Brilliant green bile broth 2%:

This medium consisted of 10 gm peptic digest of animal tissue, 10 gm lactose, 20 gm oxgall and 0.0133 gm brilliant green. Forty grams of medium were suspended in one liter distilled water, then boiled until dissolved completely.

The medium was then distributed in fermentation tubes containing inverted Durham's tubes and sterilized by autoclaving at 121°C for 15 minutes.

3.2.2 Sterilization

3.2.2.1 Sterilization of equipments:

Glassware such as petri-dishes, test tubes, mixers, pipettes and flasks were sterilized according to Grace *et al.* (1992) in an oven at 180°C for 2 hours, whereas distilled water was sterilized by autoclaving at 121°C for 15 minutes.

3.2.3 Culturing methods:

Pour plate technique for *Lactobacillus* and coliforms and surfaces plating for yeasts and moulds were used to determine the counts of bacteria during the study (Frank *et al.*, 1992).

3.2.4 Preparation of sample dilutions:

Eleven grams from a homogenated yoghurt sample were added to 99 ml of sterile distilled water at 40—45°C in a clean sterile flask, then shaken until a homogenous solution was obtained to make 10^{-1} dilution. One ml from the above-mentioned dilution (10^{-1}) was aseptically transferred to 9 ml sterile distilled water. This procedure was repeated to make serial dilutions of 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} , and 10^{-8} . From each dilution, 1 ml was transferred to duplicate petri-dishes and the culture medium was poured aseptically into each petri-dish, mixed gently, left to solidify and incubated in an inverted position. The typical colonies in each petri-dish was counted using a colony counter (Houghtby *et al.*, 1992).

3.2.5 Examination of culture:

Growth on solid media was examined visually with naked eyes for colonies appearance and change in colour.

3.2.6 Examination of bacteria:

3.2.6.1 Coliform count:

Violet red bile agar (VRBA) was used to determine the coliform count according to Christen *et al.* (1992). The plates were incubated at 32°C for 24 hours. Typical dark red colonies were counted.

3.2.6.2 *Lactobacillus bulgaricus* count:

Total *L. bulgaricus* was determined according to Frank *et al.* (1992) using MRS agar medium (MRS broth + agar). The plates were incubated at 37°C for 48 hours. Typical colonies in the selected dilution were counted.

3.2.6.3 Yeasts and moulds count:

Acidified potato dextrose agar was used to determine yeasts and moulds count according to Frank *et al.* (1992). The plates were incubated at 25°C for 5 days, and typical colonies were counted.

3.2.7 Purification of organisms:

Purification was done by sub-culturing of a well isolated typical colony on nutrient agar medium. After growth, the plates were checked by Gram's stain for purity, and it was then transferred to a plate containing a fresh solidified corresponding medium (Barrow and Feltham, 1993).

3.2.8 Identification of organisms:

The purified isolates were identified according to the criteria described by Barrow and Feltham (1993) as follows:

3.2.8.1 Primary tests:

3.2.8.1.1 Shape of the cell:

It was done by Gram's stain as described by Barrow and Feltham (1993) as follows:

Crystal violet was added to smears of young colony (18—24 hr old) on slides for one minute, followed by washing with distilled water. Iodine was added for one minute then removed with distilled water. Culture was washed with alcohol and removed. Then the slides were stained with bacteriological Gram's Saffranin for 30—60 seconds and washed with distilled water. The slides were then dried with filter paper and a drop of immersion oil was added followed by examination under the microscope.

Gram positive organisms appeared purple, while Gram negative ones appeared pink.

3.2.8.1.2 Oxidase test:

The oxidase test was performed using oxidase test paper. The colonies to be tested were removed by a platinum wire or glass rod and smeared across the surface of the oxidase test paper.

The positive reaction was shown by the development of a dark purple color within 10 seconds.

3.2.8.1.3 Catalase test:

The organisms to be tested were put on sterile slides. A drop of 3% hydrogen peroxide (H_2O_2) was added to the colony and emulsified. Evaporation of gas immediately or after 5 minutes indicated a positive result.

3.2.8.1.4 Oxidation fermentation test (OF):

Duplicate tubes of Hugh and Leifson's medium were inoculated by stabbing with a sterile straight wire. The medium in one of the tubes was covered with a layer of soft sterile paraffin oil to a depth of about one centimeter. The tubes were then incubated at $37^\circ C$ and examined daily for 14 days. Color change to yellow in both opened and covered tubes indicated fermentative organisms while the change in uncovered tube only indicated oxidative organisms. However, the negative test showed no change of color in both tubes.

3.2.8.1.5 Fermentation test:

The Brilliant green bile broth (BGBB) was used for fermentation test in coliforms according to Christen *et al.* (1992) by transferring typical colonies to tubes of BGBB broth containing Durham's tubes and incubated at $32^\circ C$ for 48 hours. The presence of gas in the inverted Durham's tubes or effervescence after gentle agitation indicated a positive test. Failure to show gas production within 48 hours indicated a negative test.

3.3 Statistical analyses:

The data were subjected to the analyses of variance (ANOVA) using the general linear model (GLM) procedure of the Statistical Analyses Systems (SAS) to determine the quality of plain yoghurt.

The samples were analyzed to determine the effect of manufacturing methods on total solids, fat, protein, solids-not-fat, titratable acidity, *Lactobacillus bulgaricus* count, coliform count and yeasts and moulds count. Means were separated using Duncan Multiple Range Test with $P \leq 0.05$.

CHAPTER FOUR

RESULTS

4.1 Chemical composition:

Table (4.1) shows the chemical composition of plain yoghurt in the two factories from which samples were collected. Total solids, fat, solids-not-fat and titratable acidity were significantly affected by factory ($P < 0.001$). The solids-not-fat (SNF) content was high in factory 1 ($10.08 \pm 0.02\%$) compared to factory 2 ($9.69 \pm 0.05\%$), while the total solids, fat and titratable acidity were higher in factory 2 ($14.57 \pm 0.10\%$, $4.88 \pm 0.07\%$ and $1.27 \pm 0.01\%$) respectively, than in factory 1 ($14.27 \pm 0.02\%$, $4.19 \pm 0.02\%$ and $0.97 \pm 0.01\%$ respectively).

The protein content was not significantly affected in both plants ($3.76 \pm 0.03\%$ and $3.75 \pm 0.03\%$ respectively).

4.2 Microbiological quality:

Table (4.2) shows the microbiological content of plain yoghurt collected from the two factories.

Lactobacillus bulgaricus, coliform bacteria and yeasts and moulds counts were not significantly affected by factory ($P > 0.05$), although the highest values were found in factory 1 ($\log_{10} 9.38 \pm 8.97$ cfu/gm, $\log_{10} 4.17 \pm 3.85$ cfu/gm and $\log_{10} 5.04 \pm 5.50$ cfu/gm respectively), than in factory 2 ($\log_{10} 9.13 \pm 8.76$, $\log_{10} 3.56 \pm 2.85$ and $\log_{10} 4.85 \pm 4.41$ cfu/gm respectively).

Table (4.1) Chemical composition of plain yoghurt as affected by factory:

Chemical composition%	Factories			
	Factory (1)	Factory (2)	G.M	S.L
Total solids	14.27 ^b ±0.02	14.57 ^a ±0.10	14.42±0.05	***
Fat	4.19 ^b ±0.02	4.88 ^a ±0.07	4.54±0.05	***
Protein	3.76 ^a ±0.03	3.75 ^a ±0.03	3.75±0.02	N.S.
SNF	10.08 ^a ±0.02	9.69 ^b ±0.05	9.89±0.03	***
Titrateable acidity	0.97 ^b ±0.01	1.27 ^a ±0.01	1.12±0.01	***

Means within each row bearing the same superscripts are not significantly different ($P>0.05$).

*** = ($P<0.001$)

N.S. = Non significant ($P>0.05$)

S.L. = Significance level

G.M.= Grand mean

Table (4.2) Microbiological quality of plain yoghurt as affected by factory:

Microbial count (log ₁₀ cfu/gm)	Factories			
	Factory (1)	Factory (2)	G.M.	S.L.
<i>L. bulgaricus</i>	9.38 ^a ±8.97	9.13 ^a ±8.76	9.26±8.74	N.S.
Coliform bacteria	4.17 ^a ±3.85	3.56 ^a ±2.85	3.87±3.55	N.S.
Yeasts and moulds	5.04 ^a ±4.50	4.85 ^a ±4.41	4.96±4.31	N.S.

Means within each row bearing the same superscripts are not significantly different (P>0.05).

S.L. = Significance level

N.S. = Non significant (P>0.05)

G.M = Grand mean

cfu = colony forming unit

4.3 Effect of storage period on the chemical composition of plain yoghurt:

Table (4.3) shows the effect of storage period on the chemical composition of plain yoghurt. The storage period did not significantly ($P>0.05$) affect total solids, fat, protein, solids-non-fat and titratable acidity. Total solids content was slightly increased from $14.40 \pm 0.15\%$ at day 1 to $14.50 \pm 0.16\%$ at day 5, then decreased to $14.37 \pm 0.15\%$ at the end of storage period.

Fat content revealed mean values of $4.51 \pm 0.30\%$, $4.57 \pm 0.36\%$ and $4.53 \pm 0.39\%$ at days 1, 5 and 10 respectively.

The protein content was gradually and steadily increased from $3.73 \pm 0.06\%$ at day 1 to $3.78 \pm 0.03\%$ at the end of storage period.

The solids-not-fat content was $9.89 \pm 0.16\%$, $9.93 \pm 0.19\%$ and $9.84 \pm 0.24\%$ at days 1, 5 and 10 respectively, while the titratable acidity gradually increased from day 1 ($1.06 \pm 0.12\%$) to $1.13 \pm 0.15\%$ at the end of storage period.

Table (4.4) presents the effect of storage period on chemical composition in each plant from which samples were collected. In factory (1) the total solids and fat contents gradually decreased as the storage period progressed. The values were $14.25 \pm 0.04\%$ and $4.21 \pm 0.03\%$ at day 1 for total solids and fat respectively, decreased to $14.22 \pm 0.05\%$ and $4.14 \pm 0.03\%$ at the end of storage period. However in the second plant the values increased from $14.54 \pm 0.18\%$ and $4.81 \pm 0.13\%$ at the beginning of storage period to $14.66 \pm 0.22\%$ and $4.93 \pm 0.14\%$ at day 5, then slightly decreased towards the end of storage period.

The protein content showed an increasing trend in the first plant and a decreasing trend in the second plant.

Table (4.3) Effect of storage period (days) on the chemical composition of plain yoghurt:

Parameter	Time of storage period(days)				
	1	5	10	G.M.	S.L.
Total solids%	14.40 ^a ± 0.15	14.50 ^a ±0.16	14.37 ^a ±0.15	14.42 ±0.04	N.S.
Fat%	4.51 ^a ±0.30	4.57 ^a ±0.36	4.53 ^a ±0.39	4.54 ±0.02	N.S.
Protein%	3.73 ^a ±0.06	3.76 ^a ±0.04	3.78 ^a ±0.03	3.76 ±0.01	N.S.
S.N.F	9.89 ^a ±0.16	9.93 ^a ± 0.19	9.84 ^a ±0.24	9.89 ±0.03	N.S.
Titrateable acidity%	1.06 ^a ±0.12	1.13 ^a ±0.15	1.19 ^a ±0.19	1.13 ±0.04	N.S.

Means within each row bearing the same superscripts are not significantly different ($P>0.05$).

N.S. = Non significant ($P>0.05$)

S.L. = Significance level

G.M. = Grand mean

Table (4.4) The chemical composition of plain yoghurt within each factory as affected by storage period (days):

Time of storage period (days)		Total solids%	Fat%	Protein%	SNF%	Titrateable acidity%
Factory (1)	1	14.25 ^{ab} ±0.04	4.21 ^a ±0.03	3.67 ^b ±0.05	10.04 ^a ±0.04	0.94 ^b ±0.01
	5	14.34 ^a ±0.02	4.21 ^a ±0.03	3.79 ^{ab} ±0.05	10.12 ^a ±0.03	0.98 ^a ±0.01
	10	14.22 ^b ±0.05	4.14 ^a ±0.03	3.81 ^a ±0.04	10.08 ^a ±0.05	1.00 ^a ±0.01
	G.M.	14.27±0.04	4.19±0.02	3.76±0.04	10.08±0.02	0.97±0.02
	S.L.	N.S.	N.S.	N.S.	N.S.	***
Factory (2)	1	14.54 ^a ±0.18	4.81 ^a ±0.13	3.79 ^a ±0.04	9.73 ^a ±0.07	1.17 ^c ±0.02
	5	14.66 ^a ±0.22	4.93 ^a ±0.14	3.72 ^a ±0.05	9.74 ^a ±0.10	1.28 ^b ±0.02
	10	14.51 ^a ±0.14	4.91 ^a ±0.11	3.75 ^a ±0.05	9.60 ^a ±0.08	1.37 ^a ±0.01
	G.M.	14.57±0.05	4.88±0.04	3.75±0.02	9.69±0.05	1.27±0.06
	S.L.	N.S.	N.S.	N.S.	N.S.	***

Means within each column bearing the same superscripts are not significantly different ($P>0.05$).

*** = ($P<0.001$)

N.S. = Non significant ($P>0.05$)

S.L. = Significance level

G.M. = Grand mean

The solids-non-fat content followed the same trend in yoghurt of two plants. The values increased to a maximum at day 5, then slightly decreased towards the end of storage period.

As the storage period progressed the titratable acidity increased in the first and second plant from $0.94 \pm 0.01\%$ and $1.17 \pm 0.02\%$ respectively at the beginning of storage to $1.0 \pm 0.01\%$ and $1.37 \pm 0.01\%$ respectively at the end of storage period.

4.5 Microbiological quality of plain yoghurt as affected by storage period (days):

Table (4.5) Showed that *L. bulgaricus* counts were not significantly affected by storage period ($P>0.05$), although, the count increased from 9.05 ± 0.69 at day 1 to 9.27 ± 0.29 at day 5, then decreased to 8.39 ± 0.46 at the end of storage period.

Coliform bacteria count was significantly affected by storage period ($P<0.05$), increasing from 1.82 ± 0.53 at the beginning to 4.31 ± 0.32 at the end of storage period.

Yeasts and moulds count significantly ($P<0.01$) increased from 3.07 ± 0.04 at day 1 to 5.41 ± 0.09 at day 10.

Table (4.6) showed that the microbiological quality of plain yoghurt was effected by the storage period in the two factories. While *L. bulgaricus* count gradually decreased with time in factory 1, the count increased to a maximum (9.57 ± 9.22) at day 5, followed by a decrease to 7.93 ± 7.37 at the end of storage period in factory 2.

However, coliform bacteria and yeasts and moulds counts gradually increased with time in the two factories. Coliform count increased from 2.35 ± 1.37 to 4.63 ± 4.31 in factory 1 and from 1.29 ± 0.46 to 4.00 ± 3.20 in factory 2. Yeasts and moulds counts increased from 3.11 ± 2.51 to 5.50 ± 4.92 in factory 1 and from 3.03 ± 2.73 to 5.32 ± 4.85 in factory 2.

Table (4.5) Microbiological quality of plain yoghurt as affected by storage period:

Time of storage period (day)	<i>L. bulgaricus</i> count (log ₁₀ cfu/gm)	Coliform count (log ₁₀ cfu/gm)	Yeasts and moulds count (log ₁₀ cfu/gm)
1	9.05 ^a ±0.69	1.82 ^b ±0.53	3.07 ^b ±0.04
5	9.27 ^a ±0.29	2.94 ^{ab} ±0.09	3.74 ^b ±0.35
10	8.39 ^a ±0.46	4.31 ^a ±0.32	5.41 ^a ±0.09
Grand mean	8.90±0.26	3.02±0.72	4.07±0.70
S.L.	N.S.	*	**

Means within each column bearing the same superscripts are not significantly different (P>0.05).

cfu = colony forming unit

* = (P<0.05)

** = (P<0.01)

S.L. = Significance level

N.S. = Non significant (P>0.05)

Table (4.6) The microbiological quality of plain yoghurt within each factory as affected by the storage period (days):

Time of storage period (day)		<i>L. bulgaricus</i> count (log ₁₀ cfu/gm)	Coliform count (log ₁₀ cfu/gm)	Yeasts and moulds count (log ₁₀ cfu/gm)
Factory (1)	1	9.75 ^a ± 9.42	2.35 ^b ± 1.37	3.11 ^b ± 2.51
	5	8.98 ^b ± 8.67	2.85 ^b ± 1.91	4.08 ^b ± 3.61
	10	8.85 ^b ± 8.82	4.63 ^a ± 4.31	5.50 ^a ± 4.92
	G.M.	9.19 ± 0.28	3.86 ± 0.69	4.23 ± 0.69
	S.L.	N.S.	**	***
Factory (2)	1	8.36 ^{ab} ± 7.84	1.29 ^b ± 0.46	3.03 ^b ± 2.73
	5	9.57 ^a ± 9.22	3.03 ^b ± 2.39	3.39 ^b ± 2.69
	10	7.93 ^b ± 7.37	4.00 ^a ± 3.20	5.32 ^a ± 4.85
	G.M.	8.62 ± 0.49	2.77 ± 0.79	3.91 ± 0.71
	S.L.	***	***	***

Means within each column bearing the same superscripts are not significantly different (P>0.05).

cfu = colony forming unit

** = (P<0.01)

*** = (P<0.001)

S.L. = Significance level

N.S. = Non significant (P>0.05)

G.M. = Grand mean

CHAPTER FIVE

DISCUSSION

The present study showed significant differences in the quality of yoghurt from two plants in Khartoum State as well as variation in the quality of yoghurt during storage period of 10 days.

The average total solids of plain yoghurt collected from factory 1 and as well as during storage period was lower than that collected from factory 2, and these results were higher than those reported by Aly *et al.* (2004) who found that total solids in plain yoghurt was 13.52%. However these results were in accordance with the findings of Omer (2003) who reported that total solids content ranged from 14% to 15.92% and Mohammed (2006) who found that total solids content gradually increased from the beginning of storage, then decreased at the end of storage period.

The average fat content of plain yoghurt and also during storage period in factory 1 was lower and ranged between 4.14%—4.21%, than in factory 2 with the range of 4.81%—4.93%. These results were higher than those reported by Younus *et al.* (2002) who found that fat content was 2.94%—3.50% and Aly *et al.* (2004) who found fat content to be 3.75%.

Protein content was 3.67%—3.81% in factory 1, while in factory 2 was 3.72%—3.79%, the results were in line with findings of Haj *et al.* (2007). However, these results are not in accordance with the findings of Aly *et al.* (2004), who reported protein content of 4.15% in plain yoghurt.

These results are also not in line with findings of Shanley (1973) who found that the protein content of yoghurt decreased with the progress of storage period.

The solids-not-fat (SNF) content was 10.04%—10.12% in factory 1, which was higher than in factory 2 (9.60%—9.74%), and this might be due to the highest level of fat in factory 2. These results were comply with

Sudanese Standards (2007) which states that the minimum solids-not-fat (SNF) content should be 8.2% and in line with the findings of Younus *et al.* (2002). However these results were lower than that reported by Haj *et al.* (2007). Tamime and Deeth (1980) reported that the content of fat, protein and ash will affect the solids-non-fat content, so it is very important to standardized the milk as well as to fix the level to acceptable standard.

The titratable acidity percent in factory 1 yoghurt was (0.94%—1.00%), which was lower than that in factory 2 (1.17%—1.37%). The results of acidity in factory 1 are in line with the findings of Salvador and Fiszman (2004) who found that titratable acidity was 0.82%—1.10% for whole yoghurt. However these results were lower than the results reported by Dalles and Kechagias (1989) who reported the acidity of commercial yoghurt range from 1.02% to 2.15%. In factory 2 the results were higher than that reported by Salvador and Fiszman (2004) and are in line with the findings of Dalles and Kechagias (1989). These results are also in line with findings of Alkali *et al.* (2007) who reported that the titratable acidity of yoghurt increased with the progress of storage period due to growth of microorganisms.

L. bulgaricus count was $\log_{10} 9.38 \pm 8.97$ cfu/gm for factory 1 yoghurt, this was higher than the mean count of *L. bulgaricus* for factory 2 yoghurt ($\log_{10} 9.13 \pm 8.76$ cfu/gm). These results are in line with the findings of Ishibashi and Shimamura (1993). Similar results were obtained by Ashraf (2006) who reported that the mean count of *Lactobacillus spp.* for recombined mixed milk yoghurt was $\log_{10} 9.53$ cfu/ml. However these results are higher than that obtained by Haj *et al.* (2007) who reported the mean of *L. bulgaricus* to be $\log_{10} 7.50$ cfu/gm. The results were in agreement with the results of Aly *et al.* (2004) who reported that the *L. bulgaricus* decreased at the end of storage period.

The coliform count in factory 1 was $\log_{10} 4.17 \pm 3.85$ cfu/gm. This results was higher than in factory 2 yoghurt ($\log_{10} 3.56 \pm 2.85$ cfu/gm), and this might be due to contamination and an unhygienic processing conditions of yoghurt. These results were higher than that reported by Tarakci and küçüköner (2003), similar for that reported by Jaffar (2006) and in agreement with Aly *et al.* (2004) who reported that the coliform count increased with the progress of storage period in plain yoghurt.

Yeasts and moulds in factory 1 was $\log_{10} 5.04$ cfu/gm, this result was higher than in factory 2 yoghurt ($\log_{10} 4.85$ cfu/gm). Thus the highest yeast and moulds count which were associated with the two plants suggesting poor standards of hygiene at these factories. These results are in line with findings of El-Makki (2006) who reported that the yeast and moulds ranged from 0 to $\log_{10} 5.42$ cfu/gm and Aly *et al.* (2004) who reported that the yeasts and moulds counts increased with the progress of storage in plain yoghurt.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion:

The results of this study concluded that the plain yoghurt in two plants in Khartoum State showed a high significant variation concerning the chemical composition.

The study revealed high coliform and yeasts and moulds counts of plain yoghurt, meaning contamination and unhygienic processing conditions.

On the other hand while the storage period had no effect on the chemical composition, the microbiological quality was affected.

6.2 Recommendations:

1. Establishment of an efficient and good quality control system during processing.
2. Standardization of milk for yoghurt manufacture should be observed to meet legal standards.
3. Authorities should frequently inspect the dairy plants to confirm that their products comply with the required standards.

REFERENCES

- Abdalla, W.M. and El Zubeir, I.E.M. (2006). Microbial hazard associated with fermented milk (roub and mish) processing in Sudan. *Int. J. Dairy Sci.*, 1 (1): 21–26.
- Abu-Tarboush, H.M. (1996). Comparison of associative growth and proteolytic activity of yoghurt starter in whole milk from camels and cows. *J. Dairy Sci.*, 79: 366—371.
- Adams, M.R. (1990). Typical aspects of fermented foods. *Trends in Food Science Technology*, 1: 141—144.
- Adams, M.R. and Moss, M.O. (1995). *Food microbiology*, Cambridge U.K. Royal Society Chemistry, P. 18, 255—265.
- Addis, E.; Fleet, G.H.; Cox, J.M.; Kolak, D. and Leung, T. (2001). The growth, properties and interactions of yeasts and bacteria associated with the maturation of camembert and blue veined cheeses. *Int. J. Food Microbial.*, 69 (1,2): 25—36.
- Adolfsson, O.; Meydani, S.N. and Russel, R.M. (2004). Yoghurt and gut function. *Am. J. Clin. Nutr.*, 50: 245—256.
- Alkali, J.S., Okonkwo, T.M., and Umoru, S.A. (2007). Effect of Thermization on shelf stability of yoghurt. *Electronic Journal of Environmental, Agricultural and Food Chemistry*. 6 (4): 1957—1964.
- Aly, S.A., Galal, E.A. and Elewa, N.A. (2004). Carrot yoghurt: sensory, chemical, microbiological properties and consumer acceptance. *Pakistan J. Nutr.*, 3 (6): 322—330.
- Anonymous, (1989). Yoghurt standard. (TS 1330). Turkish Standards Institute. Necatibey Cad. 112. Bakanliklar, Ankara, Turkey.
- AOAC (2003). *Official Methods of Analysis of AOAC International*, 17th Ed, AOAC International, Gaithersburg, MD, USA.
- Ashraf, H.A.H. (2006). Some quality aspects of set yoghurt produced from reconstituted whole and mixed milk powder. M.Sc. Thesis, University of Khartoum, Sudan.

- Barrow, G.I. and Feltham, R.K. (1993). Manual for the identification of medical bacteria. 3rd ed., Cambridge. University Press, Cambridge. U.K.
- Bille, G.P. and Keya, L.E. (2002). Comparison of some properties of vat heated and dry skim milk powder fortified set yoghurts. *J. Food Technol. Africa*, 7: 21—23.
- Binary Dictionary (2005) online@www.Binary-dictionary.com/milk 190—115.
- Boudraa, G.; Touhami, M.; Pochart, P.; Soltana, R.; Mary, J.Y. and Desjeux, J.F. (1990). Effect of feeding yoghurt versus milk in children with persistent diarrhea. *J. Paed. Gastroenterol. Nutr.*, 11: 509—512.
- Bradley, R.L.; Arnold, Jr.E.; Barbano, Jr.D.M.; Semerad, R.G.; Smith, D.E. and B.K. Vines, (1992). Chemical and physical methods. In *Standard Methods for the Examination of Dairy Products*, 16th edition, Ed., R.T. Marshall. Washington, DC: American Public Health Association, pp: 433—531.
- Campbell-platt, G. (1987). *Fermented foods of the world. A dictionary and guide*. London. Butterworths. ISBN. 0—407,313—314.
- Canganella, F.; Ovidi, M.; Paganini, S.; Vettraino, A.M.; Bevilacqua, L. and Trovatelli, L.D. (1998). Survival of undesirable microorganisms in fruit yoghurts during storage at different teperatures. *Food Microbiology*. 15: 71—77.
- Carbo, M.R.; Lanciotti, R.; Albenzio, M. and Sinigaglia, M. (2001). Occurrence and characterization of yeasts isolated from milks and dairy products of Apulia region. *Int. J. Food Microbial.*, 69: 147—152.
- Christen, G.L.; Davidson, P.M.; McAllister, J.S. and L.A. Roth, (1992). Coliform and other indicator bacteria. In *Standard Methods for the Examination of Dairy Products*, 16th edition, Ed., R.T. Marshall. Washington, DC: American Public Health Association, pp: 247—269.

- Cocolin, L.; Aggio, D.; Manzano, M.; Cantoni, C. and Comi, G. (2002).
An application of PCR-DGGE analysis to profile the yeast
populations in raw milk. *Int. Dairy J.*, 12: 407—411.
- Dalles, T. and Kechagias. C. (1989). Chemical composition of commercial
types yoghurt and changes during cold storage of yoghurt from
sheep's milk. *Deltio Ethnikis Epitropis Galaktos Ellada*. 1(1):
32—41.
- Douglas, G. (2005). Dairy Science and Technology, online @www.foodsci.uoguelph.ca/dairy.edu.
- Driessen, F.M. (1988). Bacteriën in zuursels voor gefermenteerde melk-en
zuivel producten. *Voedingsmiddelentechnologie*. 24: 22—25.
- Early, R. (1998). Liquid milk and cream. In *The Technology of Dairy
Products*, 2nd edition, Ed., R. Early. Blackie Academic and
Professional, London, U.K.
- Egmond, H.P. (1994). Aflatoxin in milk. In *The Toxicology of Aflatoxins,
Human Health, Veterinary and Agricultural Significance*, Ed.,
Eaton, D.L. and J.D. Groopman, Academic press, London, p: 365.
- Elena, M.; Ferdenandz, G.; Wenefrida, L. and Joya, M. (2004).
Development of β caroten rich juice. *Milchwissenschaft*. 19: 200—
206.
- El-Makki, J.M. (2006). Chemical and microbiological evaluation of
plain and fruit yoghurt in Khartoum State, Sudan. M.Sc. Thesis,
University of Khartoum, Sudan.
- Elson, M. and Hass, M.D. (2005). Dairy products online@www.health.net/scr/article.asp.
- Fleet, G.H. (1990). Yeasts in dairy products, *J. Appl. Bacteriol.*, 68: 199—
211.
- Fleet, G.H. (1992). Spoilage yeasts. *Crit. Rev. Biotech.*, 12: 1—44.
- Frank, J.F.; Christen, G.L. and L.B. Bullerman, (1992). Tests for groups of
microorganisms. In *Standard Methods for the Examination of Dairy
Products*, 16th edition, Ed., R.T. Marshall. Washington, DC:
American Public Health Association, pp: 271—286.

- Fox, P.F. and McSweeney, P.L.H. (1998). Dairy chemistry and biochemistry. Blackie Academic and Professional, London, U.K.
- Gadaga, T.H.; Mutukumira, A.N. and Narvhus, J.A. (2000). Enumeration and identification of yeasts isolated from Zimbabwean traditional fermented milk. *Int. Dairy J.*, 10: 459—466.
- Gadaga, T.H.; Mutukumira, A.N. and Narvhus, J.A. (2001). The growth and interaction of yeasts and lactic acid bacteria isolated from Zimbabwean naturally fermented milk in UHT milk. *Int. Food Microbial.*, 68: 21—32.
- Galvano, F.; Vittori, G. and Giacamo, G. (2000). Occurrence and stability of aflatoxin M₁ in milk and milk products. A world-wide review. *J. Food Prot.*, 59: 1079—1090.
- Grace, V.; Houghtby, G.A.; Rudnick, H.; Whaley, K. and J. Lindamood, (1992). Sampling dairy and related products. In *Standard Methods for the Examination of Dairy Products*, 16th edition, Ed., R.T. Marshall. Washington, DC: American Public Health Association, pp: 59—83.
- Haj, M.H.M., El Owni, O.A.O. and El Zubeir, I.E.M. (2007). Assessment of chemical and microbiological quality of stirred yoghurt in Khartoum State. *Res. J. Anim. Vet. Sci.*, 2: 56—60.
- Hartman, P.A. and Hartman, P.S. (1976). Coliform analyses at 30 °C. *J. Milk Food Technol.*, 39: 763—767.
- Hassan, A.N. and Frank, J.F. (2001). Starter cultures and their use. In Marth E.H and Steele. J.L. *Applied Dairy Microbiology*. Marcel Dekker, New York, 151—206.
- Hassanin, N.I. (1999). Stability of aflatoxin M₁ during manufacture and storage of yoghurt-yoghurt cheese. *J. Food Prot.*, 48: 67—73.
- Heyman, M. (2000). Effect of lactic acid bacteria on diarrheal diseases. *J. Am. Coll. Nutr.*, 19: 137—146.
- Houghtby, G.A.; Maturin, L.J. and E.K. Koenig, (1992). Microbiological count methods. In *Standard Methods for the Examination of Dairy Products*, 16th edition, Ed., R.T. Marshall. Washington, DC: American Public Health Association, pp: 213—246.

- Hutkins, R.W. (2001). Metabolism of starter cultures. In Marth, E.H and Steele, J.L. *Applied Dairy Microbiology*, Marcel Dekker, New York, 207—241.
- Ibrahim, M.K.E.; EL-Abd, M.M.; Mehriz, A.M. and Ramadan, F.A.M. (1992). Effect of some stabilizers and sweetening materials on the properties of a new ice cream-like yoghurt. *Egyptian, J. Dairy Sci.*, 20(1): 67— 81.
- Ishibashi, N. and Shimamura, S. (1993). Bifidobacteria: Research and development in Japan. *Food Technol.*, 47: 126— 135.
- James, S. and Stratford, M. (2003). Spoilage yeast with emphasis on the genus *Zygosaccharomyces*. In yeasts in food. Edited by B.T and R.V. Behrs Verlag. 171—1191. PP.
- Jensen, J.P. and Hausler, W.J. (1975). Productivity of boild and autoclaved violet red bile agar. *J. Milk Food Prot.*, 38: 279—280.
- Kivanc, M. (1992). Public health significance of moulds and mycotoxins in fermented dairy products. *Nahrung*. 36: 578—583.
- Kurt, A. (1981). Süt teknolojisi. Atatürk Üniversitesi Yayinlari, no: 573. Atatürk Üniversitesi Basim Evi.
- Lankes, H.; Ozer, H.B. and Robinson, R.K. (1998). The effect of elevated milk solids and incubation temperature on the physical properties of natural yoghurt. *Milchwissenschaft*. 53: 510—513.
- Law, A.J. (1996). Effect of heat treatment and acidification on the dissociation of bovine casein micelles. *J. Dairy Res.*, 63: 35—48.
- Lee, Y.S; Morr, V.C. and Seo, A. (1990). Comparison of milk-based and soy milk-based yoghurt. *J. Food Sci.*, 55(2): 532—535.
- Loureiro, V. and Querol, A. (1999). The prevalence and control of spoilage yeasts in foods and beverages. *Trends Food Sci. Technol.* 10: 156—165.
- Lovell, R.H. (1990). *Dairy microbiology* 2nd edition. Robinson, K.R. Editor, London and New York, 250 —270.

- Lucey, J.A. and Singh, H. (1998). Formation and physical properties of acid milk gels. *Areview Food Res. Int.*, 30: 529—542.
- Marshall, V.M. (1993). Starter cultures for milk fermentation and their characteristics. *J. Soc. Dairy Technol.*, 46: 49—56.
- Marranzini, R.M. (1987). The effect of threonine and glycine levels on threonine aldolase activity of yoghurt microorganisms grown in a modified-milk medium. Master thesis. University of Florida, Gainesville, FL.
- Marranzini, R.M.; Schmidt, R.H.; Shireman, R.B.; Marshall, M.R. and Cornell, J.A. (1989). Effect of threonine and glycine concentrations on threonine aldolase activity of yoghurt microorganisms during growth in a modified milk prepared by ultrafiltration. *J. Dairy Sc.*, 72: 1142—1148.
- Marth, E.H. and Steele, J.L. (1998). *Applied dairy microbiology*. Marcel Dekker, Inc., New York.
- Matalon, M.E. and Sandine, W.E. (1986), *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and yoghurt. *Areview. Cult. Dairy Prod. J.*, 21: 6—12.
- Mayoral, M.B.; Martin, R.; Sanz, A.; Hernandez, P.E.; Gonzalez, I. and Garcia, T. (2005). Detection of *Kluyveromyces marxianus* and other spoilage yeasts in yoghurt using a PCR-culture technique. *Int. J. Food Microbial.*, 105: 27—34.
- Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, PM., Shaprio, C. and Tauxe, V. (1999). Food-related illness and death in the United States. *Emerging Infectious Diseases*, 5: 607—625.
- Mishra, H. and Das, C. (2003). A review on biological control and metabolism of aflatoxins. *Crit, Rev. Food Sci. Nutr.*, 43: 245—264.
- Mohammed, H.M.H. (2006). Assessment of chemical and microbiological quality of stirred yoghurt in Khartoum State, M.Sc. Thesis, University of Khartoum, Sudan.
- Moreira, S.R.; Schwan, R.F.; Carvalho, E.P. and Wheals, A.E. (2001). Isolation and identification of yeasts and filamentous fungi from yoghurts in Brazil. *Introduction. Brazilian J. Microb.*, 32: 117—122.

- Morgan, D., Nawman, C.P. Hutchinson, D.N., walkes, A.M., Rowe, B. and Maijd, F. (1993). Verotoxin-producing *Escherichia coli* O157:H7 infections associated with the consumption of yoghurt. *Epidemiology and Infection*, 111: 181—187.
- Omer, S.H.M. (2003). Study of chemical and physical properties of yoghurt at Khartoum Dairy Product Company (KDPC). M. Sc. Thesis, University of Khartoum. Sudan.
- Pearce, R.J. (1995). Food functionality: success or failure for dairy based ingredients. *Aust. J. Dairy Technol.*, 50: 15—23.
- Pitt, J.I. and Hocking, A.D. (1997). *Fungi and food spoilage*. Blackie Academic and professional, New York.
- Rajagopal, S.N. and Sandine, W.E. (1990). Associative growth and properties of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in skim milk. *J. Dairy Sci.*, 73, 894.
- Rasmussen, H.L. (1981). Enumeration, identification of cultured product organisms. *Dairy Food and Sanitation*. 1(7): 286—289.
- Restaino, L.; Bills, S.; Tscherneff, K. and Lenovich, L.M. (1983). Growth characteristics of *Saccharomyces rouxii* isolated from chocolate syrup. *Appl. Environ. Microbiol.* 45: 1614—1621.
- Robison, R.K. (1981). Properties of yoghurt and their appraisal. *Dairy Indust. Int.* 46: 31—35.
- Robinson, R.K. and Tamime, A.Y. (1990). Microbiology of fermented milks. In *The Microbiology of Milk Products*, 2nd edition, Ed., R.K. Robinson. Elsevier Appl. Sci., London, pp: 291—345.
- Roostita. R. and Fleet, G.H. (1996). Growth of yeasts in milk and associated changes to milk composition. *Int. J. Food Microbiol.*, 31: 205—219.
- Roy, U.; Batish, V.; Grover, S. and Neelakantan, (1996). Production of antifungal substances by *Lactococcus lactis* ssp *Lactis*. *Int. J. Food Microbiol.*, 32: 27—34.

- Rybka, S. and Kailasapathy, K. (1995). The survival of culture bacteria in fresh and freeze-dried AB yoghurt. *Aust. J. Dairy Technol.*, 50(2): 51—57.
- Salvador, A. and Fiszman, S.M. (2004). Textural and sensory characteristics of whole and skimmed flavored set-type yoghurt during long storage. *J. Dairy Sci.*, 87(12): 4033—4041.
- Schmidt, R.H.; Kennedy, L.B.; McMullen, E.B. and Mason, E.R. (1989). Survey of the inhibitory effects of glycine on threonin aldolase activity of yoghurt microorganisms. *J. Agric. Food Chem.*, 37(5): 1215—1216.
- Shanley, M.K. (1973). Analysis of free sugars in yoghurt. *Aus. J. Dairy Technol.*, 28(2): 58—60.
- Shibario, S.; Upadhaya, M. and Toivonen, P. (1998). Yoghurt and shelf life time extending. *J. Hort. Sci. Biotec.*, 73: 862—866.
- Spreer, E. (1998). *Milk and dairy product technology*. Marcel Dekker, Inc. New York, NY.
- Staffe, M.C. (1998). *Cultured milk and fresh cheeses. The technology of dairy products*. Blakie Academic, London. 123—153.
- Stanley, (1998). Microbiology of fermented milk products, in Early, R. *The technology of dairy products*, Blackie Academic London, 123—157.
- Sudanese Standard and Metrology (2007). *Yoghurt*. Sudanese Standards and Metrology Organization (SSMO). No., 291.
- Takano, T. (1998). Milk derived peptides and hypertension reduction. *Int. Dairy J.*, 8: 375—381.
- Tamime, A.Y. and Deeth, H.C. (1980). Yoghurt: technology and biochemistry. *J. Food Prot.*, 43: 939—977.
- Tamime, A.Y. and Robinson, R.K. (1985). *Yoghurt-Science and Technology*. Pergamon Press, Oxford.
- Tamime, A.Y. and Robinson, R.K. (1991). *Yoghurt science and technology*. CRC press, New York, NY.

- Tamime, A.Y. and Robinson, R.K. (1999). Yoghurt science and technology, 2nd edition. CRC Press LLC, Boca Raton, FL.
- Tamime, A.Y. and Marshall, V.M. (1997). Microbiology and Biochemistry of cheese and fermented milk, 2nd edition, edited by Law, B. Chapman and Hall, London. PP. 57—152.
- Tarakçi, Z. and küçüköner, E. (2003). Physical, chemical, microbiological and sensory characteristics of some fruit-flavored yoghurt. YYÜ Vet Fak Derg., 14 (2): 10—14.
- Taylor, G.R.J. and Williams, C.M. (1998). Effects of probiotics and prebiotics on blood lipids. Br. J. Nutr., 80: 225—230.
- Tetra Pak, A.B. (1995). Dairy Processing Handbook. Published by Tetra Pak, A.B. Processing Systems A.B. S-221 86 Lund, Sweden.
- van't Veer, P.; Dekker, J.M.; Lamers, J.W.J.; Kok, F.J.; Schouten, E.G.; Brants, H.A.M.; Sturmans, F. and Jhermus, R.J. (1989). Consumption of fermented milk products and breast cancer. A case-control study in the Netherlands. Cancer Res., 49: 4020—4023.
- Vedamuthu, E.R. (1991). The yoghurt story-past, present and future. Dairy Food and Environmental Sanitation. 11 (4): 202—203.
- Vesa, T.H.; Marteau, P.H.; Zidi, S.; Briet, F.S.; Pochart, P.H. And Rambaud, J.C. (1996). Digestion and tolerance of lactose from yoghurt and different semi-solid fermented dairy products containing *Lactobacillus acidophilus* and Bifidobacteria in lactose maldigesters-Is bacterial lactase important ? Eur. J. Clin. Nutr., 50; 730—733.
- Viljoen, B.C. and Greyling, T. (1995). Yeasts associated with Cheddar and Gouda making. Int. J. Food Microbiol., 28: 79—88.
- Viljoen, B.C. (2001). The interaction between yeasts and bacteria in dairy environments. Int. J. Food Microbiol. 63: 37—44.
- Viljoen, B.C.; Lourens-Hattingh, A.; Ikalafeng, B. and Peter, G. (2003). Temperature abuse initiating yeast growth in yoghurt. Food Res. Int., 36: 193—197.

- Walstra, P.; Geurts, T.J.; Noomen, A.; Jellema, A. and VanBoekel, MA.J.S. (1999). Dairy technology. Principles of milk properties and processes. Marcel Dekker, New york.
- Wikipedia (2005) online@www.cu.wikipedia.org/pasteurization.
- Wilkins, D.W.; Schmidt, R.H. and Kennedy, L.B. (1986a). Threonine aldolase activity in yoghurt bacteria as determined by headspace gas chromatography. J. Agric. Food Chem., 34 (1): 150—152.
- Wilkins, D.W.; Schmidt, R.H.; Shireman, R.B.; Smith, K.L. and Jezeski, J.J. (1986b). Evaluation acetaldehyde synthesis from L-[14 C 9 U] threonine by *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. J. Dairy Sci., 69: 1219—1224.
- Younus, S.; Masud, T. and Aziz. T. (2002). Quality evaluation of market Yoghurt/Dahi. Pak. J. Nutr., 1 (5): 226—230.